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Case Report

Severe chronic osteomyelitis caused by *Morganella morganii* with high population diversity

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SUMMARY

A case of chronic osteomyelitis probably caused by *Morganella morganii*, occurring over a period of 30 years, is reported. The organism was identified through a combination of sample culture, direct sequencing, and 16S RNA gene amplicon sequencing. Further whole-genome sequencing and population structure analysis of the isolates from the patient showed the bacterial population to be highly diverse. This case provides a valuable example of a long-term infection caused by an opportunistic pathogen, *M. morganii*, with high diversity, which might evolve during replication within the host.

1. Introduction

Morganella morganii is typically prevalent in the environment and in the human intestinal tract as a commensal organism. On rare occasions, it can act as an important opportunistic pathogen, causing infection among postoperative, immunocompromised, and intensive care unit patients. *M. morganii* can be isolated from patients with several types of infection; however, only a few cases with bone involvement have been reported in the literature.[1–4]

A case of severe chronic osteomyelitis caused by *M. morganii* and lasting for approximately 30 years in the patient is reported here. After radical debridement followed by skeletal fixation and antibiotic therapy, the osteomyelitis was cured without recurrence. Further analysis of the bacterial diversity by genomic sequencing of multiple colonies revealed that the bacterial population was highly diverse, supporting the diverse-community model of bacteria during chronic infection suggested in recent studies.[5–7] This case provides a valuable example of a long-term infection caused by an opportunistic pathogen, *M. morganii*, with high diversity, which might evolve during replication within the host.[8]

2. Case report

A 44-year-old man was admitted with a high fever and wound pain, with ulceration and odorous liquid exudation from his left distal thigh. He reported a trauma to the left leg caused by falling from a car approximately 30 years ago during childhood. He had not received treatment, and the affected area had become swollen a few days later. He had developed pain, a high fever, and coma, and nearly died. After receiving treatment at a local hospital involving simple surgery (the details were unknown), his symptoms improved, but a sinus tract near the surgical site emerged with pus effusion, which occasionally self-closed. Over the past 30 years, the patient experienced spasticity in the left knee, and the left lower limb was gradually shortening, but weight-bearing was unaffected. During this period, the patient occasionally experienced pain and ulceration with pale yellow pus oozing

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around the affected area accompanied by a high fever. After antibiotic treatment (data unavailable), his body temperature returned to normal and the skin ulceration area healed. This situation recurred two to three times annually. Thirteen years ago, the patient had gradually developed a limp and the left thigh showed various deformities.

Laboratory tests of the patient's blood showed a white blood cell count of 6.1×10^9 cells/l (normal range 4.0–10.0), erythrocyte sedimentation rate (ESR) of 15 mm/h (normal range 0–30), and C-reactive protein (CRP) level of 6.5 mg/l (normal range 0.3–8). A physical examination revealed that the left limb was 5 cm shorter than the right limb. The femur in the upper left knee joint showed inward angulation and a sinus tract was observed inside the upper left knee with odorous pus effusion. An additional closed sinus tract outside of the upper left knee was observed. Bacterial cultures of the purulent liquid were positive for *M. morganii*. Plain radiographic examination revealed a deformity in the left distal femur and the potential existence of dead bone. Sinus angiography showed that this sinus tract was connected with a bony sequestrum and a very large cavity with a volume of approximately 56.8 mm^3 around the sequestrum (Figure 1A). Three-dimensional reconstruction of computed tomography images revealed disappearance of the left knee joint space, pronation of the left distal femur, and the presence of a large section of dead bone in the medullary cavity (data not shown).

The patient underwent excision of the sinus tract and debridement of the medullary masses (Figure 1B, C). Intraoperative findings, pathology, and culture revealed an abscess caused by *M. morganii*. After skeletal fixation of the left knee joint and left femur, he was given therapy with closed lavage and negative pressure drainage for 3 days and was treated with 1 g intravenous imipenem every 12 h for 5 weeks. Although his markers increased upon completing the operation, the wound gradually closed, and inflammation markers such as the white blood cell count, CRP, and ESR decreased to normal ranges 1 month later. The external fixation was removed 3 months later and the osteomyelitis has not recurred.

3. Methods

To preclude contamination during culture and comprehensively explore the pathogen responsible for this 30-year chronic infection, clinical samples and isolates were further investigated by meta-genome sequencing, 16S RNA amplicon sequencing, and whole-genome sequencing. To identify the pathogen causing this infection with an unbiased comprehensive approach, next-generation DNA sequencing (NGS) was used to directly determine all DNA sequences present in the clinical samples obtained from this patient.

16S RNA gene amplicon combined with deep sequencing is less expensive and time-consuming than NGS. Therefore, 16S RNA gene amplification with primers (forward: AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGTAYTGGGYDTAAAGNG; reverse: CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTATCACGCCTACNVGGGTATCTAATCC) and subsequent deep sequencing was used to determine whether similar results could be achieved.

To further characterize this patient's clinical isolates, 10 colonies of *M. morganii* were selected randomly and subjected to whole-genome sequencing. Briefly, genomic DNA was extracted using the QIAamp DNA Micro Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Genomic libraries were

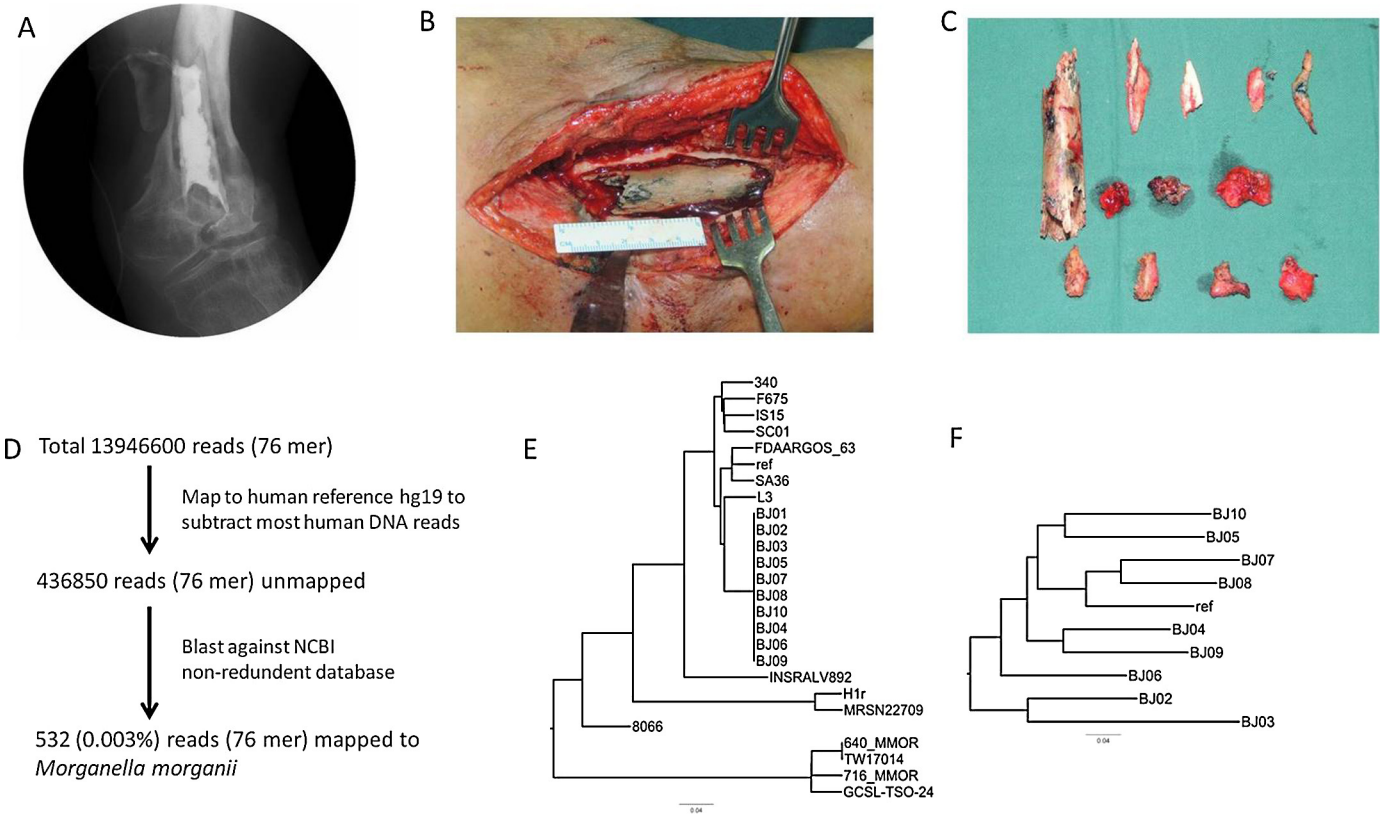

Figure 1. (A) Sinus angiography showing the appearance of a bony sequestrum. (B) (C) Views of dead bones surrounded by normal bones and soft tissues: a total of 12 dead bones were excised from this site, with the longest being approximately 8.35 cm. (D) Detection of potential pathogens by whole-genome sequencing: schematic representation of the direct sequencing of clinical samples. (E) (F) Maximum likelihood dendrogram for 10 clinical isolates together with 16 publically available *Morganella morganii* genomes or 10 clinical isolates, supporting the diverse-community model of the bacterial population during chronic infection.

prepared using the Illumina-compatible Epicentre Nextera DNA Sample Prep Kit (Epicentre, Chicago, IL, USA), and libraries were sequenced on the MiSeq 50 platform (Illumina, San Diego, CA, USA). De novo genome assembly was performed using the SOAPdenovo2 assembler.[9] Gene prediction and annotation was conducted using RASTtk.[10] Average nucleotide identity analysis was conducted using BLAST ANIb analysis.[11] Assembly visualization was performed using BRIG 0.95.[12] Both SAMtools (v0.1.18) were used for single nucleotide polymorphism (SNP) calling, and the concatenated sequences of all SNPs from the 10 isolates were used to generate a maximum parsimony (MP) phylogeny.

4. Results

BLAST searching for all reads acquired from the meta-genome sequencing showed that 552 reads (0.003% of a total of 13 946 600 reads) were identified as *M. morganii* (Figure 1D). Surprisingly, direct sequencing of the 16S RNA gene amplicon from clinical samples indicated that *M. morganii* was the causative pathogen (data not shown).

Whole-genome sequencing of 10 *M. morganii* colonies revealed a high degree of identity among colonies compared to the reference genome, *Morganella morganii* subsp. *morganii* KT (**Supplementary Material**, Figure S1). Average nucleotide identity analysis confirmed this result (**Supplementary Material**, Table S1). These sequencing data were further parsed to extract only SNPs that were of high quality in all genomes. Concatenated SNPs generated against the reference genome were used to construct a maximum likelihood tree of 10 clinical isolates and all 16 publically available *M. morganii* genomes (Figure 1E). The results showed that the clinical isolates clustered on the same branch and were distant from other strains.

Further analysis of the diversity of these 10 strains, using BJ01 as the reference, revealed a deeply branched phylogeny, indicating the coexistence of diverse sublineages (Figure 1F). In total, 53 mutations were identified in some but not all isolates and only three mutations shared by all isolates, suggesting that most mutations that arose during the course of infection did not fix, with sites remaining polymorphic within the patient. To quantify the diversity, the numbers of pairwise SNPs within each isolate were determined. Among these isolates, the median number of pairwise SNPs was 4, with a maximum of 8.

5. Discussion

Chronic osteomyelitis is typically caused by *Staphylococcus aureus* and other Gram-negative bacilli and is a relapsing and persistent infection of the bones in patients who have suffered from open trauma.[13,14] Lower levels of antibiotics in the bone than in the serum lead to biofilm formation and poor vascular supply, typically resulting in the failure of antibiotic therapy.[15] Thus, surgical debridement of the infected site and long-term antibiotic treatment is necessary to cure chronic osteomyelitis.[15,16] In the patient case presented here, poverty and inadequate treatment at a rural trauma center meant that the left femur was not adequately treated and the patient suffered consistent relapses of infection.

This patient represents an unusual case. First, this man had suffered for the past 30 years, likely because of relapsing infection due to the lack of thorough therapy for his infection. The severe trauma sustained 30 years previously, although without open wounds, and the subsequent infection had nearly killed this man. Each year since then, he had received limited treatment to alleviate the symptoms, but he did not undergo thorough debridement and optimal antibiotic therapy. Second, the section of dead bone was as long as 8.35 cm, and there were 11 small sections. These necrotic bones remained in situ for many years, and thus they may have formed during childhood and persisted for 30 years. The volume of the cavity surrounding the dead bone was as large as 56.8 mm^3. This large cavity may have provided an ideal growth environment for microorganisms to propagate and diffuse. Third, bone or joint infections caused by *M. morganii* are uncommon, particularly in chronic osteomyelitis.

M. morganii is distributed widely in the environment and is commonly found in the intestinal tract of healthy individuals; thus it is typically considered part of the normal flora and an opportunistic pathogen.[17,18] This pathogen infrequently causes orthopedic disease and has only been reported in a few cases of sporadic septic arthritis. In a recent retrospective study involving 109 patients examined for 10 years, age, one or more comorbidities, and community-acquired infection via the urinary or hepatobiliary tract were the main predisposing factors for *M. morganii* infection. Acute Physiology and Chronic Health Evaluation II (APACHE II) scores were the only significant risk factor for mortality.[19] This bacterium has been isolated in clinical samples from patients with septic arthritis,[1–4,20–24] meningitis,[25] chorioamnionitis,[26] pericarditis,[27] orbital abscess,[28] and hepatic hydatid cyst,[29] among others.

This case showed that the opportunistic pathogen *M. morganii* can cause osteomyelitis and remain within the host for many years. Recent studies have shown that bacteria causing chronic bacterial infectious diseases acquire numerous adaptive mutations under the within-host pressure of natural selection and evolve to establish diverse populations.[5,18] Therefore, it is possible that in this case, the *M. morganii* strains accumulated large numbers of mutations over the past 30 years and were highly diverse in their population structure. The present data support this hypothesis, showing that the infecting *M. morganii* lineages were genotypically diverse in this osteomyelitis infection. This finding that the bacterial population of a chronic infection, even in a single person, is highly diverse, strongly supports the diverse-community model.[18] Diversity may induce the pathogenicity of bacterial populations by enhancing their adaptive ability in response to host pressure.[6] Therefore, in addition to inadequate treatment, the high diversity of the *M. morganii* strains within this patient may explain why this opportunistic pathogen induced a severe osteomyelitis.

Author contributions

WFL and FB designed the study; JLZ, HFL, LF, YHK, RGY, LY, LL, RYL, and MSL performed the experiments and data analysis. This manuscript was written by YHK, WFL, and FB.

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Conflict of interest: There are no conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijid.2016.07.016.

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